Thrombosis Research 141 (2016) 49–57

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Standardization of a well-controlled in vivo mouse model of thrombus formation induced by mechanical injury

Chaojun Tang [a,*,1], Yinyan Wang [a,1], Daoxi Lei [b], Lu Huang [b], Guixue Wang [b], Qingjia Chi [b], Yiming Zheng [b], Christian Gachet [c], Pierre H. Mangin [c], Li Zhu [a]

[a] Cyrus Tang Hematology Center, MOH Key Lab of Thrombosis and Hemostasis, Jiangsu Key Lab of Preventive and Translational Medicine for Geriatric Diseases, Jiangsu Institute of Hematology, Soochow University, Suzhou, China
[b] Key Laboratory of Biorheological Science and Technology, Ministry of Education, Bioengineering College of Chongqing University, Chongqing, China
[c] Unité Mixte de Recherche (UMR) S949, Institut National de la Santé et de la Recherche Médicale (INSERM), Fédération de médecine translationelle (FMTS) de Strasbourg, Université de Strasbourg, Etablissement Français du Sang (EFS)-Alsace, Strasbourg, France

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 12 February 2016
Accepted 29 February 2016
Available online 2 March 2016

Keywords:
Mechanical injury
Thrombosis
Animal model
Anti-thrombotic

ABSTRACT

Objective: Vascular plug formation by mechanical injury that exposes abundant extracellular matrix is an ideal model to mimic thrombus formation. The objective of this study was to standardize our previously established in vivo mouse model of thrombus formation induced by mechanical injury.
Results: The mechanical injury was exerted by pinching the abdominal aorta with hemostatic forceps for either 15 (moderate injury) or 60 (severe injury) seconds. Thrombus formation was monitored for 20 min in real time using a fluorescent microscope coupled to a CCD camera. In the moderate injury, thrombus formation peaked at approximately 1 min after injury and resolved within 3 min, with the mean AUC (area under the curve) of 165.2 ± 17.29 mm^2, whereas a larger thrombus was observed upon the severe injury, with the mean AUC of 600.5 ± 37.77 mm^2. Using scanning electron microscopy and HE staining, a complete deformation of the endothelium in the moderate injury model and the exposure of the media in the severe injury model were observed. The model was also evaluate for its application on the effects of antithrombotic drugs targeting GP IIb–IIIa (eptifibatide), ADP receptor $P2Y_1$ (MRS2500) and $P2Y_{12}$ (clopidogrel), and thrombin (hirudin) on thrombus formation.
Conclusions: We have improved a vascular injury model with optimal reproducibility and feasibility that allows evaluating the effect of anti-thrombotic drugs on thrombus formation in vivo.

1. Introduction

Vessel wall injury triggers acute platelet activation and plug formation, followed by coagulant activity and the formation of fibrin-containing thrombi at the site of injury. Although these events are crucial for limiting blood loss at sites of tissue trauma, they may also block diseased vessels, leading to ischemia and infarction of vital organs. Experimental models that enable real-time in vivo observation of arterial thrombus formation in mice help to elucidate the mechanisms and to facilitate the discovery of new targets for antithrombotic modalities. Several mouse models of thrombosis that mimic human vascular disease have been developed using various methods [1], such as chemical injury induced by ferric chloride ($FeCl_3$) in the carotid artery [2–4], laser injury to the cremaster or mesenteric arteries [5–10] or mechanical injury through arterial compression [11–16], ligation [17] or introduction of a guide wire [18,19] and balloon [20,21].

Mechanical injury that exposes abundant extracellular matrix is an ideal model to mimic thrombosis allowing evaluating a series of targets for the anti-platelet therapy. Mechanical injury through compression by forceps was first described to induce thrombus formation in the guinea pig [11,12]. Then Pierangeli developed a compression injury on the femoral vein in mice [13,14], while Nieswandt group tried an occlusive thrombosis model by the compression injury on the carotid artery in mice [15,16]. We have recently established a mechanical injury model by forceps-induced compression on aorta and used in our recent studies to evaluate the interaction between collagen and GPVI [22], vWF and GPIb [23], and laminin and its receptor $\alpha_6\beta_1$ [24]. However, this compression-induced thrombosis model has not been well described for its underlying mechanism and not widely used due to the lack of a standardization. In this article, we standardized this model with severe or moderate injuries, analyzed the dynamics of thrombus formation in

Abbreviations: $DIOC_6$, 3,3′-dihexyloxacarbocyanine iodide; CCD, Charge Coupled Device; AUC, area under curve; SEM, scanning electron microscopy; $FeCl_3$, ferric chloride; MRS 2500, (1*R**,2*S**)-4-[2-iodo-6-(methylamino)-9H-purin-9-yl]-2-(phosphonooxy) bicyclo [3.1.0] hexa-ne-1-methanol dihydrogen phosphate ester tetraammonium salt.

* Corresponding author at: Cyrus Tang Hematology Center, Soochow University, Suzhou, China.
E-mail address: zjtang@suda.edu.cn (C. Tang).
[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.thromres.2016.02.032
0049-3848/

real-time, and investigated histological changes on injured vascular wall. Additionally, the applicability of this model was evaluated by measuring the effects of anti-platelet and antithrombotic drugs on thrombus formation in vivo.

2. Materials and methods

2.1. Materials

Xylazine (Rompun®) and ketamine (Imalgen®) were provided by Bayer (Puteaux, France) and Merial (Lyon, France), respectively. 3,3′-dihexylcarbocyanine iodide ($DIOC_6$) was purchased from Molecular Probes (Eugene, OR, USA). Glutaraldehyde was purchased from Electron Microscopy Sciences (Hatfield, PA). Hematoxylin and eosin (HE) staining Kit was provided by Jiancheng Bioengineering Institute (Nanjing, China) and Van Gieson (VG) staining kit was from Haling Biotechnology co., LTD (Shanghai, China). The $P2Y_1$ antagonist MRS 2500 was purchased from Tocris Bioscience (Bristol, BS11 0QL, United Kingdom), and aspirin was from Sigma (St Louis, MO, USA). The GPIIb–IIIa antagonist eptifibatide (Integrilin®) and $P2Y_{12}$ antagonist clopidogrel were obtained from Millennium Pharma (San Francisco, CA, USA), and hirudin was obtained from Transgene (Illkirch-Graffenstaden).

2.2. Mice

C57BL/6 wild-type mice (males aged 65–75 days and females aged 75–85 days) with an average body weight of approximately 25 g were chosen for this experiment. All animals were housed in a specific pathogen-free facility and all experiments approved by the University Committee on Animal Care of Soochow University (20140431).

2.3. Forceps-mediated injury procedure

Mice were anesthetized via intraperitoneal injection of ketamine (100 mg/kg) and xylazine (20 mg/kg) and the aorta was exposed (Fig. 1A). The fluorescent dye $DIOC_6$ was dissolved in DMSO at 50 mM as a stock solution, diluted in 1/500 in saline, and injected via the jugular vein (100 μl/20 g). Two degrees of mechanical injury were exerted upon the aorta by pinching with hemostatic forceps (Ultra Fine Hemostats with curved and smooth tip without jaws; tip width = 0.6 mm, 12031-21, Fine Science Tools, F.S.T./Canada) for either 15 or 60 s. After removal of the forceps, thrombus formation was monitored immediately in real time using a fluorescent microscope coupled to a CCD camera. Subsequent analyses were performed using Metamorph software. The forceps comprised four serrated jaws that were used to clamp the aorta and numbered from 1 to 4, according to the degree of injury (Fig. 1B). For the moderate injury, the forceps were compressed on the aorta to fixed position 1 for 15 s to crush the vascular wall. The crush was performed using the nearly rectangular contact surface of the forceps. For the severe injury, the aorta was pinched at fixed position 4 for 60 s to exert a severe crush. Fig. 1C and D shows the contact area of the forceps that we applied on the vessels.

2.4. Analysis of thrombosis

Thrombus formation was visualized in real time using a fluorescent microscope (Macrofluo®, objective 5.0×/0.5 NA, Leica Microsystems, Rueil-Malmaison, France). Fluorescent images were acquired sequentially (1 image/2 s) for 20 min using a CoolSNAP HQ2 camera (Roper Scientific, Evry, France) controlled by Metaview software (version 7, Universal Imaging, Ypsilanti, MI, USA). The thrombus surface area was quantified at each time point using Metamorph software (version 7, Universal Imaging), and the area under the curve (AUC) was calculated using GraphPad software (Prism 5.0) and expressed as μm [2] * 20 min. A region of interest was applied for each thrombus, and a threshold was determined for individual experiments.

2.5. Histological analysis of the injury site on the vessel wall

Injuries were induced as described above. Intact arteries without injury served as controls. In order to observe both the injured vessel wall and the thrombus formation, we observed 5 min after injury and

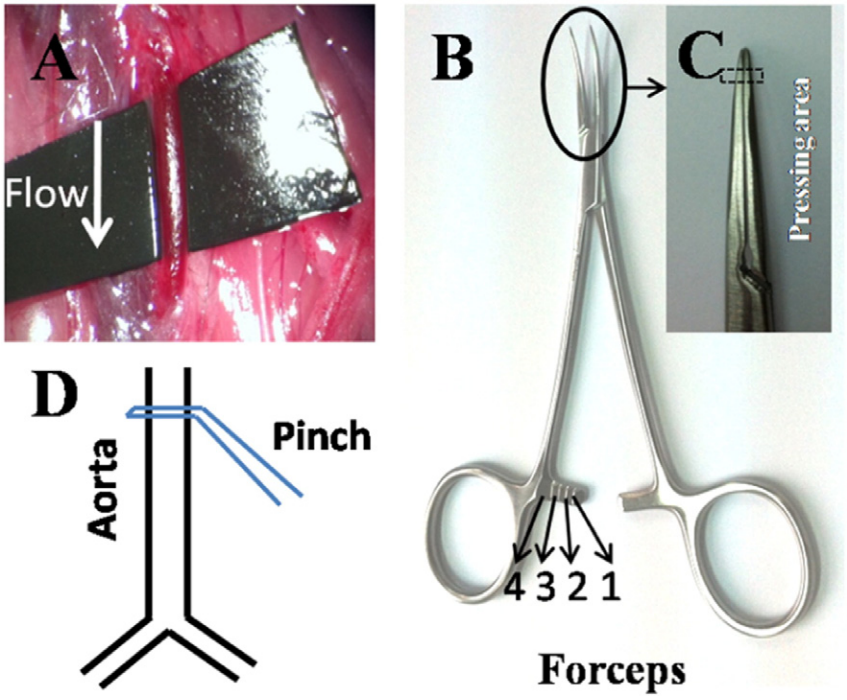

Fig. 1. Forceps-mediated injury. A and D, The aorta was separated from the vein after anesthesia and laparotomy. The injury was induced by using the forceps to pinch the aorta. B, The forceps used for pinching. The degree of injury was controlled using different buckle settings as follows: 1 for moderate injury, 4 for severe injury. C, The pressing area of the forceps was indicated.